

A new frog species of the subgenus *Asperomantis* (Anura, Mantellidae, *Gephyromantis*) from the Bealanana District of northern Madagascar

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Abstract

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A recent study on a group of rough-skinned *Gephyromantis* frogs from Madagascar (Anura: Mantellidae: Mantellinae) established a new subgenus, *Asperomantis*, with five described species and one undescribed candidate species. Based on newly collected material from the Bealanana District, we address the taxonomy of this candidate species, and reveal that it consists of two populations with low genetic and morphological divergence but considerable bioacoustic differences that are obvious to the human ear. As a result, we describe some of the specimens formerly assigned to *Gephyromantis* sp. Ca28 as *G. angano* **sp. n.** and assign the remaining specimens from a locality between Bealanana and Antsohihy to a new Unconfirmed Candidate Species, *G. sp. Ca29*. *Gephyromantis angano* **sp. n.** is a small species that strongly resembles *G. asper* and *G. ceratophrys*, but it differs from these and all other *Gephyromantis* species by a unique, clinking advertisement call. The new species may be highly threatened by habitat fragmentation, but at present we recommend it be treated as Data Deficient until more data are available to assess its distribution. We discuss the curious relationship between *G. angano* **sp. n.** and *G. sp. Ca29*, which we suspect may represent a case of incipient speciation. We also identify two additional new Unconfirmed Candidate Species of *Gephyromantis* based on sequence data from other specimens collected during our surveys in the Bealanana District.

Introduction

Madagascar's 317 nominal frog species belong to six families: Mantellidae Laurent, 1946 (213 species), Microhylidae Günther, 1858 (91 species), Hyperoliidae Laurent, 1943 (11 species), Ptychadenidae Dubois, 1987 (1 species), Dicroglossidae Anderson, 1871 (1 species, introduced), and Bufonidae Gray, 1825 (1

species, introduced) (AmphibiaWeb 2017). Mantellidae is the island's most diverse radiation, and among the amphibians, the only family-level unit wholly endemic to Madagascar and the nearby Comoros (two undescribed species are found on nearby Mayotte; Vences et al. 2003). It is divided into three subfamilies, of which the Mantellinae Laurent, 1946 is the most diverse, comprising 129 species in seven genera. The most diverse of

these genera is *Gephyromantis* Methuen, 1920, with 42 nominal species.

Gephyromantis is currently divided into six subgenera: *Asperomantis* Vences, Köhler, Pabijan, Bletz, Gehring, Hawlitschek, Rakotoarison, Ratsavina, Andreone, Crotini & Glaw, 2017, *Duboisimantis* Glaw & Vences, 2006, *Gephyromantis* Methuen, 1920, *Laurentomantis* Dubois, 1980, *Phylacomantis* Glaw & Vences, 1994, and *Vatomantis* Glaw & Vences, 2006 (Kaffenberger et al. 2012, Vences et al. 2017). In their recent study, Vences et al. (2017) erected the subgenus *Asperomantis* for the *Gephyromantis asper* clade, which contains five nominal species: *G. ambohitra* (Vences & Glaw, 2001), *G. asper* (Boulenger, 1882), *G. ceratophrys* (Ahl, 1929), *G. tahoitra* Glaw, Köhler & Vences, 2011, and *G. spinifer* (Blommers-Schlösser & Blanc, 1991). A sixth species that was identified by Perl et al. (2014), *G. sp. Ca28*, clearly belongs to this subgenus as well based on its morphology and genetic affinities (Vences et al. 2017). It was originally detected based on DNA sequences of a specimen from ‘Antsahan’i Ledy’, and later two additional specimens from a site between Bealanana and Antsohihy were added to it (Vences et al. 2017), but no adult male specimen or bioacoustic data have been available until now.

Here, we address the taxonomy of *G. sp. Ca28* using an integrative taxonomic approach based on bioacoustics, morphology, morphometrics, and genetics, from new material collected between December 2015 and January 2016. We also provide additional sequence data and new localities for a selection of *Gephyromantis* species encountered during the collection of the new species.

Materials and methods

Fieldwork was conducted at two sites: Ampotsidy mountains, near Beandrazona (14.410–14.432°S, 48.710–48.727°E) in the Bealanana District of the Sofia Region between the 17th of December 2015 and 9th of January 2016; and in several small forest fragments near the southern border of the Bealanana District (14.701–14.758°S, 48.493–48.587°E) between the 13th and 17th of January 2016. These two locations are separated by ca. 40 km.

Specimens were captured by hand, euthanized using MS222, fixed in ~90% ethanol, and kept thereafter in 75% ethanol. Prior to fixation, a piece of muscle from the thigh was taken as a tissue sample for subsequent DNA analysis, deposited in 99% ethanol. Field numbers refer to Mark D. Scherz (MSZC), Miguel Vences (ZCMV), and David R. Vieites (DRV). Institutional abbreviations are: ZSM (Zoologische Staatssammlung München), and UADBA-A (Université d’Antananarivo Département de Biologie Animale, Amphibiens).

Call recordings were made with a Sennheiser KE66+K6 super-cardioid microphone on a Marantz PMD 661 MKII field recorder, at 44.1 kHz sampling. Bioacoustic analysis was performed in COOL EDIT PRO. Frequency information was obtained through Fast Fourier Transformations

(FFT; width 1024 points). Spectrograms were obtained with a Hanning window of 512-bands resolution. Temporal measurements are given as mean \pm standard deviation with range in parentheses. Terminology in call descriptions follows the call-centred terminology of Köhler et al. (2017). Recordings are deposited in the Animal Sound Archive of the Museum für Naturkunde, Berlin and are provided as Supplementary materials 1–4.

We analysed a segment of the mitochondrial DNA (mtDNA) 16S rRNA gene (*16S*). We used a salt-extraction protocol to extract DNA from tissue samples as described by Kaffenberger et al. (2012). We PCR-amplified the *16S* segment used as standard for barcoding Madagascan amphibians (Vences et al. 2005, Vieites et al. 2009) with the primer pairs AC16SAR-L/AC16SBR-H (Crotini et al. 2014) and 16SFrogL1/16SFrogH1 (Vences et al. 2010). Purification of PCR product was done using Exonuclease I and Shrimp Alkaline Phosphatase digestion. Amplicons were sequenced using the BigDye v. 3.1 cycle sequencing chemistry on a 3130xl genetic analyser (Applied Biosystems). Assembly and quality-checking was performed in CODONCODE ALIGNER v. 3.0.3 (CodonCode Corporation). Newly generated sequences were deposited in GenBank under accession numbers MF768444–MF768467.

Comparative sequences were retrieved from Vieites et al. (2009) and Vences et al. (2017), for almost all known *Gephyromantis* species including candidate species, for a total of 123 terminals. We aligned sequences using the ClustalW algorithm in MEGA7 (Kumar et al. 2016), with *Boophis madagascariensis* (Mantellidae: Boophinae) and *Guibemantis liber* (Mantellidae: Mantellinae) as hierarchical outgroups. Gaps were treated as missing data. Due to the short length of the alignment, hypervariable regions were not removed. Uncorrected pairwise distances (p-distances) between and within species in the *16S* dataset were calculated using MEGA7.

Phylogenies were calculated using Bayesian Inference (BI) in MRBAYES v. 3.2.6 (Ronquist et al. 2012) under the JC69 model in order to reduce the risk of over-parameterisation with our small dataset. The Markov chain Monte Carlo sampling included two runs of four chains each (three heated, one cold) sampled every 10^3 generations for a total of 10^6 generations. The first 25% of samples were discarded as burn-in. Parameter convergence was assessed in TRACER v 1.5 (Rambaut and Drummond 2007). For comparative purposes, we ran a maximum likelihood (ML) tree in RAXML (Stamatakis 2014) with 500 bootstrap replicates under the GTR+G model.

Morphometrics of the new material were obtained for comparison primarily with values reported by Vences et al. (2017). Measurements were taken by MV to the nearest 0.1 mm with a precision calliper, for the following characters (reiterated verbatim from Vences et al. 2017): snout–vent length (SVL), maximum head width (HW), head length from posterior maxillary commissure to snout tip (HL), horizontal eye diameter (ED), horizontal tympanum diameter (TD), distance from eye to nostril

(END), distance from nostril to snout tip (NSD), distance between nostrils (NND), foot length (FOL), foot length including tarsus (FOTL), hindlimb length from cloaca to tip of longest toe (HIL), forelimb length from axilla to tip of longest finger (FORL), length and width of femoral gland (FGL, FGW), and number of femoral gland granules (FGG) given as left/right. Webbing formulae follow Blommers-Schlösser (1979); femoral gland terminology follows Glaw et al. (2000).

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Results

During fieldwork in Ampotsidy we encountered several *Gephyromantis* species. Most notable among these was an abundant species of the subgenus *Asperomantis*, with a characteristic, high pitched, clinking call. Later, during fieldwork ca. 40 km SSW, near the road between Bealanana and Antsohihy in a forest patch locally called Andranonafindra, we encountered another relatively abundant *Asperomantis* with a lower, rasping call, similar to that called '*Gephyromantis* sp. aff. *ambohitra*' in Vences et al. (2006). Genetically the former population is assignable to the first specimen of *G.* sp. Ca28 from Antsahan'i Ledy, whereas the latter is assignable to the specimens from between Antsohihy and Bealanana (very near Andranonafindra) added to this candidate species by Vences et al. (2017) (genetics are discussed and displayed in more detail below). We investigated whether these specimens represented one or two species using an integrative approach based on bioacoustics, morphology, and molecular phylogenetics.

Bioacoustics

Advertisement calls of the *Asperomantis* species from Ampotsidy and from Andranonafindra exhibited strong and clear differences in call parameters. To illustrate these differences, we here describe these calls:

Ampotsidy: Based on call voucher ZSM 68/2016 (MSZC 0172): Calls recorded at 22h40 on the 8th of January, 2016, 50 cm above the ground on a fern above a muddy spring in primary rainforest, calling as part of a

small chorus, at 14.41949°S, 48.71938°E, 1340 m a.s.l., at an estimated air temperature between 15 and 20°C (Fig. 1, Suppl. material 1). Call series are composed of 9–25 (mean 16.7 ± 6.5 , $n = 6$) rapidly emitted, short (call duration 41–98 ms, mean $= 59.4 \pm 10.4$ ms, $n = 27$), unpulsed, tonal, single-note calls (call series duration 2798–5917 ms, $n = 6$), with silent inter-call intervals of 148–239 ms (mean 193 ± 22 ms, $n = 25$). The inter-series intervals are highly variable (26.0–64.2 s, 41.5 ± 20 s, $n = 3$). Before some call series, one or two pairs of calls are released, here termed 'warm-up calls', which we here do not consider as part of a call series, as some other series do not include these. The silent interval between warm-up calls and main call series is 1233–1335 ms ($n = 2$). The call series is amplitude modulated, with the initial few calls being of considerably lower amplitude than the subsequent calls, followed by calls at constant amplitude until the end of the series. Dominant frequency of calls is 3803 ± 59 Hz (3703–3875 Hz, $n = 6$), with a 90% bandwidth from ca. 3700 to ca. 4050 Hz. Other calls recorded were not vouchered, but shared these parameters (Suppl. material 2). Calls highly similar to the human ear in frequency and structure were heard on a daily basis from numerous individuals across several sites up to three kilometres from the coordinates of this specimen during the three week observation period, sometimes in extremely motivated, dense choruses. We infer these calls to be typical advertisement calls as they occurred both in isolation and in dense choruses, and no other calls were heard from these frogs.

Andranonafindra: Based on call voucher ZSM 58/2016 (MSZC 0196): Calls recorded at 18h40 on the 14th of January, 2016 on a broad fleshy leaf 4 m from a slow stream in degraded primary rainforest, calling as part of a large chorus, at 14.73600°S, 48.54831°E, 1180 m a.s.l., at an estimated air temperature of 17–23°C (Fig. 2, Suppl. material 3). Call series are ill-defined, composed of an indefinite number of rapidly emitted, short (call duration 51–96 ms, 59.8 ± 13 ms, $n = 10$), single-note calls, each of which is highly pulsed containing 16–21 pulses (mean 18.2 ± 1.4 , $n = 10$), the maxima of which are separated by 2–3 ms (mean 2.8 ms ± 0.4 , $n = 10$). Calls are generally separated by inter-call intervals of 210–268 ms duration (mean 226.3 ± 16.3 , $n = 10$, silence of these intervals is inferred as background calls and noise in the recording make it difficult to be certain of silence). Each call is amplitude modulated, with greatest energy at the beginning, decreasing toward the end of the call, but the call series shows no pattern of modulation (Fig. 2). Dominant frequency of calls is 3703 ± 0 Hz ($n = 10$), with a 90% bandwidth from ca. 1560 to ca. 3800 Hz. Calls of a second vouchered specimen, ZSM 59/2016 (MSZC 0203), from the same locality, had the same structure as ZSM 58/2016, but were shorter (16–68 ms, 44.0 ± 15.4 ms, $n = 10$), had slightly fewer pulses (3–19, mean 11.5 ± 4.9 , $n = 10$), and had shorter inter-call intervals (169.0 ± 53 ms, 97–243 ms, $n = 10$), but roughly the same dominant frequency (3716 ± 86 Hz, 3617–3875 ms, $n = 10$) (Suppl. material 4).

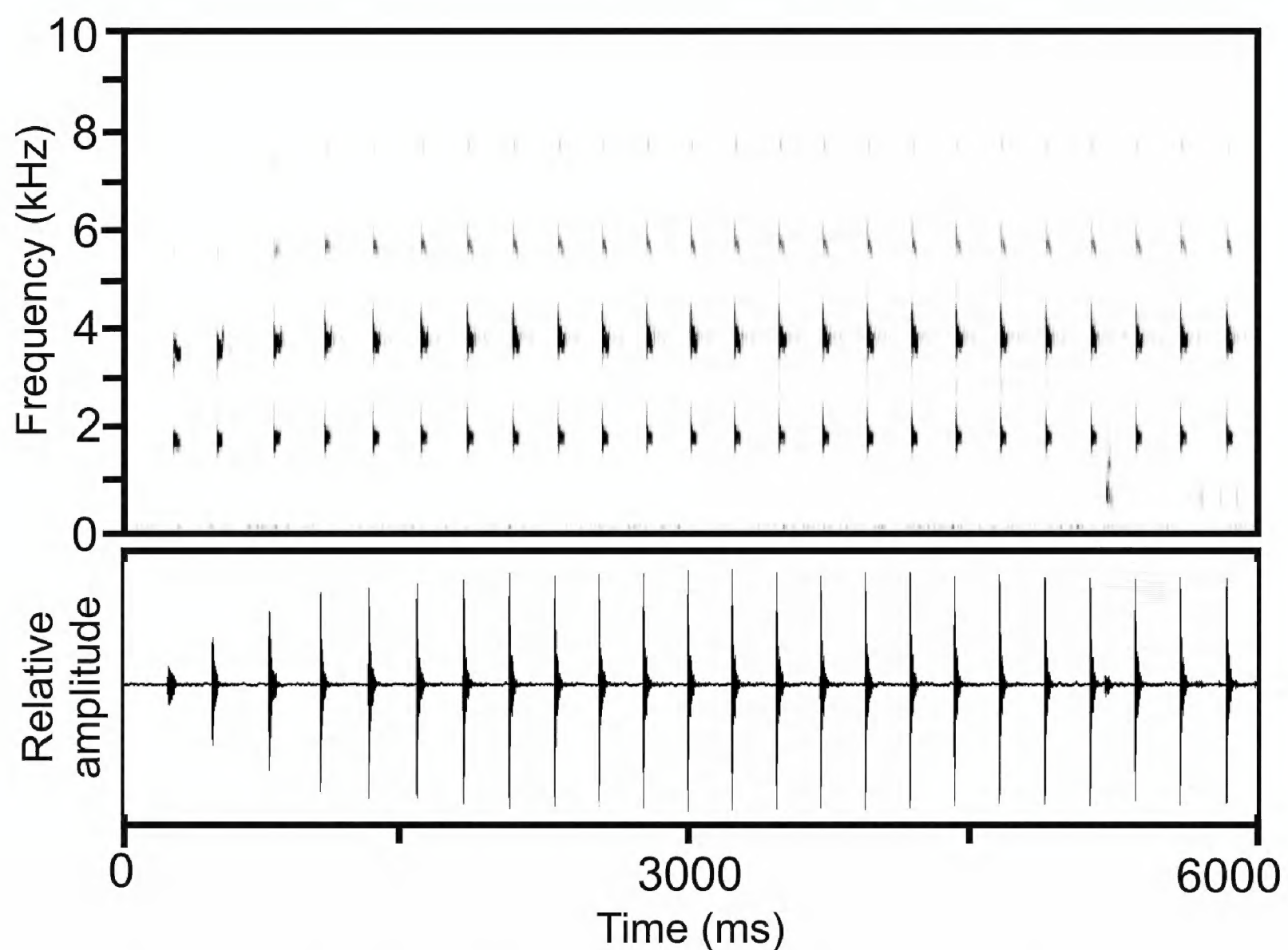


Figure 1. Spectrogram (above) and oscillogram (below) of a call series of the holotype of *Gephyromantis angano* sp. n., ZSM 68/2016 (MSZC 0172) from Ampotsidy. For the conditions of the call, see the text. The call is provided in Suppl. material 1.

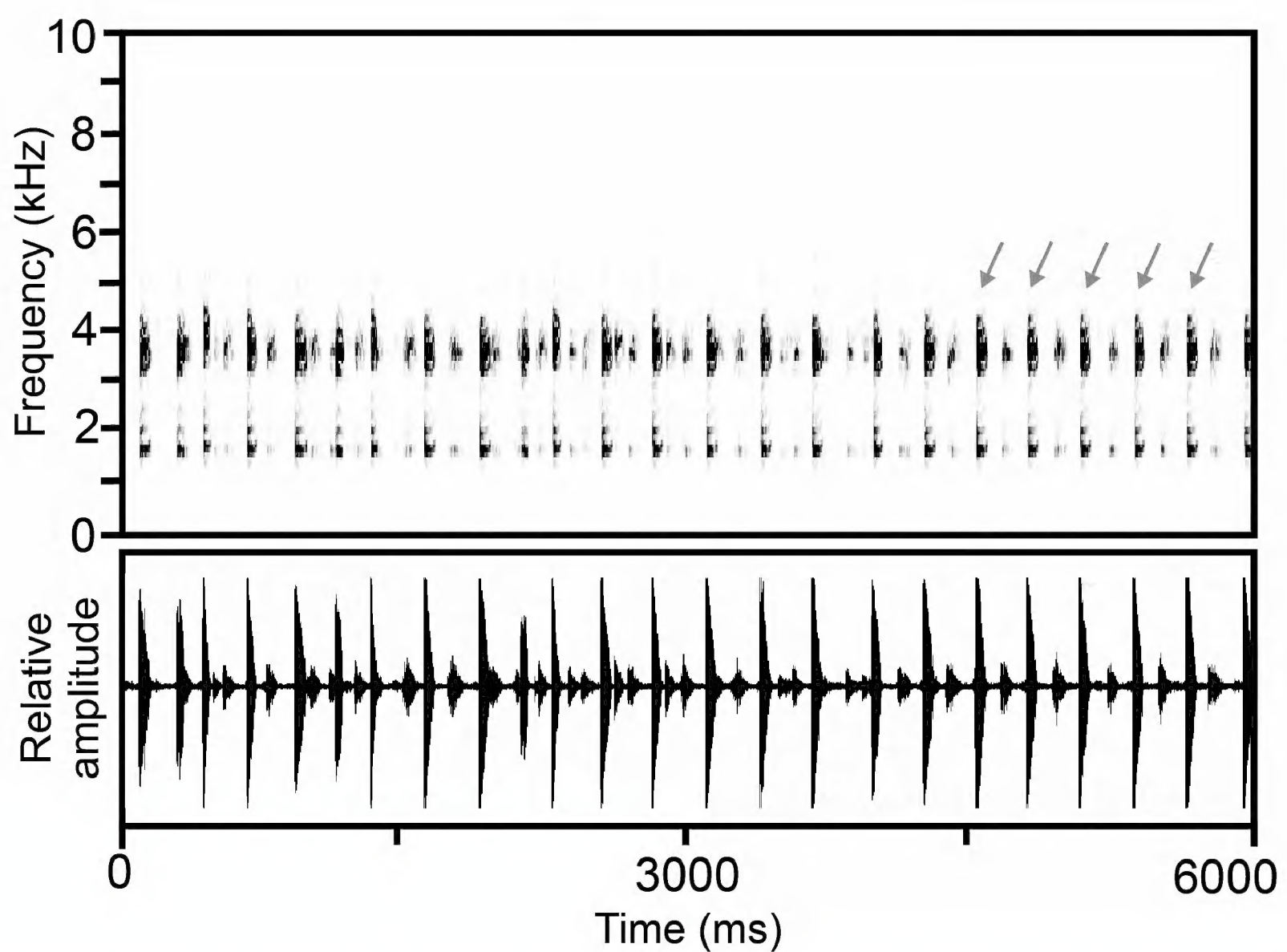


Figure 2. Spectrogram (above) and oscillogram (below) of a part of a long call series of *Gephyromantis* sp. Ca29 (ZSM 58/2016 = MSZC 0196) from Andranonafindra. For the conditions of the call, see the text. Note that in between the calls of the main recorded male (closest to the microphone), other males can be heard. In the second half of the spectrogram, five calls of the male closest to the microphone are marked with small arrows. The call is provided in Suppl. material 3.

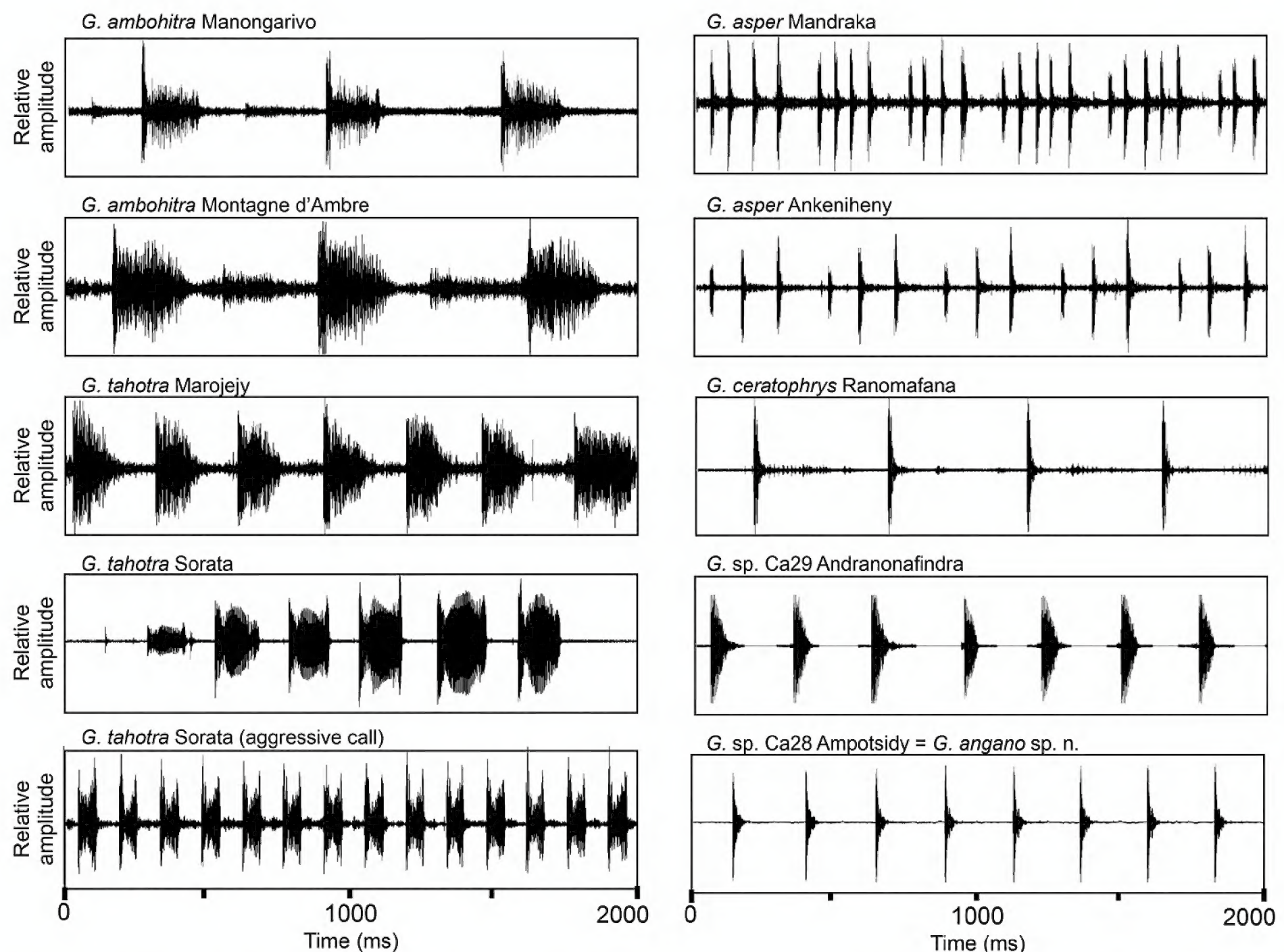


Figure 3. Bioacoustic differences among *Gephyromantis* (*Asperomantis*) species, as evident from oscillograms of their calls, adapted from Vences et al. (2017) in comparison with *Gephyromantis angano* sp. n. and *Gephyromantis* sp. Ca29.

Calls highly similar to the human ear in frequency and structure were heard on two non-consecutive nights in the vicinity of the recorded specimens during the brief survey period in this area of forest, but were not heard in other nearby patches of forest in between these nights. However, no suitable habitats (slow-flowing, shallow water) were found in the other surveyed forest patches, so their absence was anticipated. We suppose these calls also to be advertisement calls as the circumstances under which they were recorded were similar to those under which specimens from Ampotsidy were observed, but the observation period was admittedly much shorter and the sample size is small.

The advertisement calls of both of these populations are distinct from all other *Asperomantis* species (see Fig. 3, and compare data provided in Vences et al. 2017). By their short call duration, they are slightly similar to calls of *G. asper* and *G. ceratophrys*, but differ by longer note duration (mean 59.4 ± 10.4 ms vs. mean range 10.3–28.6 ms in *G. asper* and *G. ceratophrys*), by being evenly spaced (rather than arranged in fast call groups as in *G. asper*), and apparently by being more rapidly repeated than in *G. ceratophrys* (Fig. 3).

The vocalizations from Ampotsidy and Andranonafindra are also different from one another, especially in that specimens from Ampotsidy emit a tonal, unpulsed call in a clearly defined call series, whereas specimens from Andranonafindra emit a rough, strongly pulsed call without clear call series formulation. The sound impression of calls from both populations is very different to the human ear, mostly as a result of the tonal calls of specimens from Ampotsidy as opposed to the pulsed calls of specimens from Andranonafindra, although their temporal parameters are remarkably similar (call duration 59.4 ± 10.4 ms vs. 59.8 ± 13 ms; inter-call interval duration 193 ± 22 ms vs. 226.3 ± 16.3 ms). Thus the measured differences are smaller than those between other species in the subgenus *Asperomantis* (Fig. 3), but the calls are as distinguishable from one another. It is possible that the different calls represent two separate call types of the same species, as is known from *G. tahotra* (Glaw et al. 2011; Fig. 3; discussed below). However, in either location only one of the respective call types was heard, unlike in *G. tahotra*, where both call types can be heard simultaneously or independently in a single population and switching among calls appears to occur sporadically (e.g. within a single evening).

Morphology and colouration

Morphological measurements are given in Table 1. The newly collected adults are smaller than most other *Asperomantis* material, resembling in size primarily *G. asper* and *G. ceratophrys*. Male specimens from Ampotsidy and Andranonafindra are highly similar, and the only notable difference is body size (SVL 29.1–29.6 mm [n = 2] in Ampotsidy vs. 30.6–32.7 mm [n = 2] in Andranonafindra). There is a slight difference in the shape of the femoral glands, with those of specimens from Ampotsidy being slightly longer relative to their width than those of specimens from Andranonafindra. The number of femoral gland granules between the two species overlaps, being exceptionally variable in specimens from Ampotsidy with 26–69 granules per gland, whereas specimens from Andranonafindra have 42–49 (note that not all values are reported in Table 1 as some specimens from which granule number could be counted from photos were not available for measurements). Females were not available from Andranonafindra. All other measurements do not differ. The colouration of specimens is also similar (see photos in life below), and it must be noted that colouration is highly variable in all *Asperomantis* species (Vences et al. 2017). In summary, the morphological differences between these populations are on par with both inter- and intraspecific variation of other *Asperomantis* species (Vences et al. 2017).

Molecular phylogenetics

We produced new *16S* DNA sequences for 20 specimens. Our *16S* alignment of these and 103 other terminals contained 619 characters and a total of 283 variable sites, of which 241 were parsimony informative (excluding outgroups). BI and ML phylogenies of the *16S* alignment agreed in topology of the *Asperomantis* subgenus (Fig. 4), with small differences in other subgenera throughout the genus *Gephyromantis* (Suppl. material 5); the overall tree topology agrees well

with more comprehensive multi-gene studies (Kaffenberger et al. 2012). Support for the BI tree was generally high, whereas support for the ML phylogeny was rather low (Fig. 4, Suppl. material 5). Uncorrected pairwise distances (p-distances) are given for *Asperomantis* in Table 2 and for all other *Gephyromantis* species in Suppl. material 6. Specimens from Ampotsidy and Andranonafindra belong to four or five species: *G. sp.* Ca28 (one or two species; discussed in the next paragraph), *G. tahotra*, *G. horridus* (3% divergent from other *G. horridus* and recovered with negligible support as closer related to *G. ranjomavo* in our tree, so requiring closer investigation), and two divergent lineages well over 3% genetically divergent from all other *Gephyromantis* species, identified here as Unconfirmed Candidate Species sensu Vieites et al. (2009) for the first time, *G. sp.* Ca30 (a *Duboisimantis* with affinities to *G. tandroka*, separated by 6.4% uncorrected p-distance) and *G. sp.* Ca31 (a *Phylacomantis* with affinities to *G. azzurrae*, separated by 5.5% uncorrected p-distance; Suppl. material 6).

Populations of *Gephyromantis sp.* Ca28 from Ampotsidy and Andranonafindra are genetically assortative; specimens from Ampotsidy cluster with a specimen from Antsahan’i Ledy, while specimens from Andranonafindra cluster with specimens from between Antsohihy and Bealanana (Fig. 4; the names *G. angano sp. n.* and *G. sp.* Ca29 for these two populations are used pre-emptively here and in Table 1 and Fig. 3). These clades have an uncorrected p-distance between them of 1.0–3.0% (Table 1). This distance is below the typical threshold of genetic distance used to identify candidate species based on *16S* DNA barcode sequence data (Vieites et al. 2009), and agrees with intraspecific distances among other species of *Asperomantis* (see Table 2). Note that the intraspecific variation in *G. ambohitra* is exceptionally high as a result of the distance between its two clades (Fig. 4) of 5.6–7.1%; distances within these clades are 0.4–2.1% (data not shown). On the

Table 1. Morphological measurements of *Gephyromantis angano sp. n.* (formerly *G. sp.* Ca28) from Ampotsidy and Antsahan’i Ledy, and *G. sp.* Ca29 from Andranonafindra, plus two newly collected specimens of *G. tahotra* from Ampotsidy. All measurements are given in mm. Measurement abbreviations are given in the Materials and methods. The bolded specimen is the holotype of the new species described below.

Species	Field number	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	HAL	HIL	FOTL	FOL	TIBL	FGG	FGL	FGW
<i>G. angano</i> (Ampotsidy)	MSZC 0172	M	29.6	10.4	11.0	2.7	3.8	3.0	1.8	2.8	19.1	9.3	53.7	23.3	15.8	17.2	36/44	6.6	3.0
<i>G. angano</i> (Ampotsidy)	MSZC 0021	M	29.1	9.9	11.0	2.5	3.7	3.2	1.7	2.4	18.3	8.8	50.2	22.3	15.2	16.0	30/26	6.2	2.4
<i>G. angano</i> (Ampotsidy)	MSZC 0112	F	30.5	9.8	11.4	2.3	3.8	3.3	1.9	2.4	20.0	9.6	54.3	23.7	15.9	17.8	n/a	absent	absent
<i>G. angano</i> (Antsahan’i Ledy)	ZSM 1731/2010	F	26.2	8.8	10.4	2.2	3.4	2.8	1.4	2.2	18.1	8.4	50.1	21.6	15.6	15.5	n/a	absent	absent
<i>G. sp.</i> Ca29 (Andranonafindra)	MSZC 0203	M	30.6	10.5	13.1	2.4	4.0	3.5	1.7	2.6	18.7	9.0	54.3	23.8	16.0	17.4	48/47	6.2	3.1
<i>G. sp.</i> Ca29 (Andranonafindra)	MSZC 0196	M	32.7	10.7	12.0	3.0	4.0	3.3	2.0	2.7	20.3	9.9	54.9	24.6	16.8	17.5	42/49	6.1	2.9
<i>G. tahotra</i> (Ampotsidy)	MSZC 0142	M	32.0	11.9	12.2	2.5	4.3	3.2	2.0	2.8	19.8	10.5	59.4	26.1	18.0	19.0	7/3	indistinct	indistinct
<i>G. tahotra</i> (Ampotsidy)	MSZC 0148	M	33.4	11.7	12.9	3.1	4.3	3.4	2.4	3.0	20.1	10.8	60.0	26.2	18.1	19.3	22/22	indistinct	indistinct

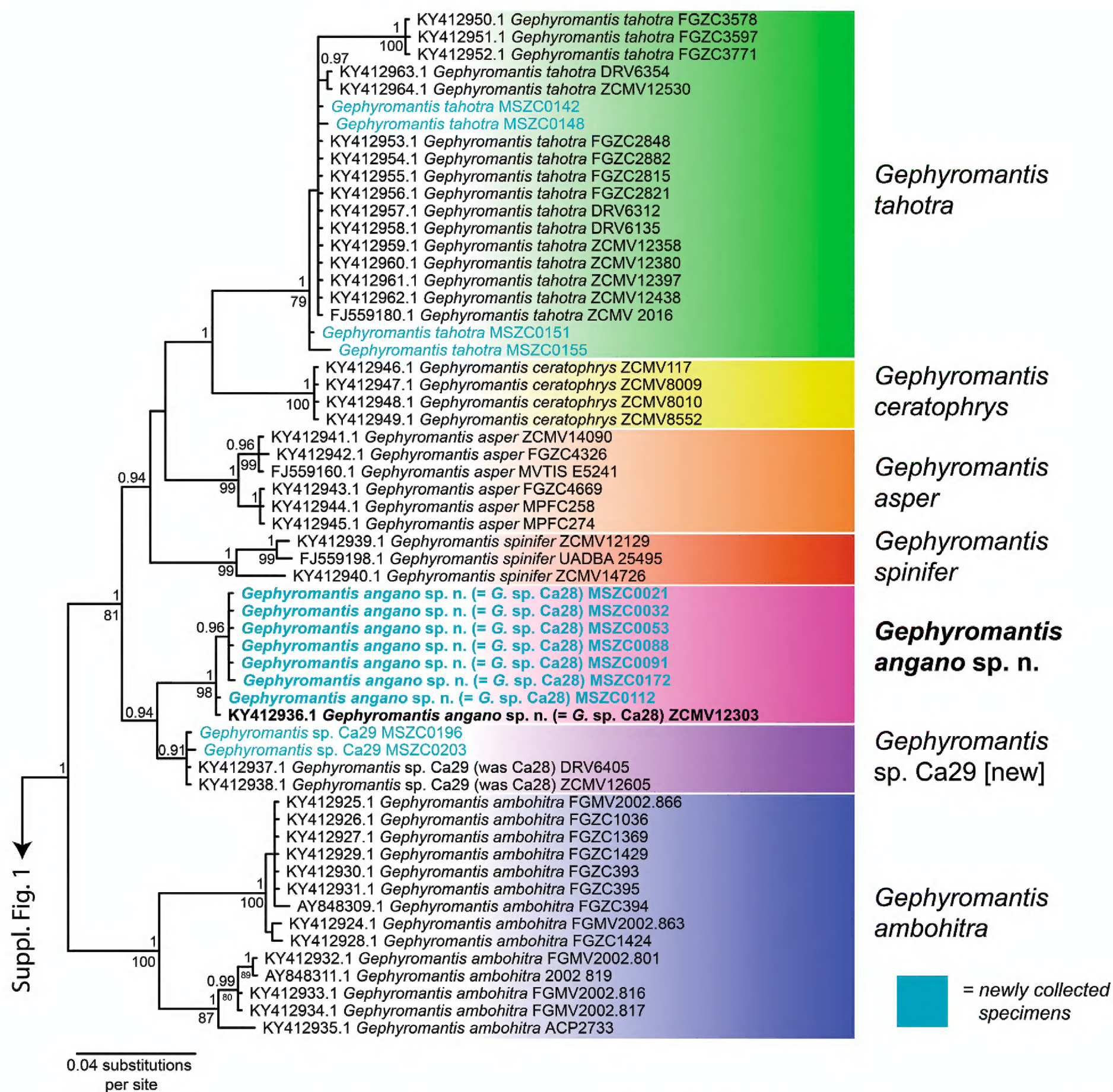


Figure 4. Phylogenetic relationships of the subgenus *Asperomantis*, reconstructed by Bayesian Inference analysis of a fragment of the mitochondrial 16S rRNA gene. Numbers above nodes denote Bayesian Posterior Probability (PP) from Bayesian Inference analysis; numbers below nodes indicate bootstrap support (%) from Maximum Likelihood analysis. PP lower than 0.9 and bootstrap support lower than 70% are not shown. Other *Gephyromantis* and outgroups are shown in Suppl. material 5. Numbers before taxon names are GenBank numbers; numbers after taxon names are field numbers.

other hand, the Ampotsidy and Andranonafindra sequences differ from all described *Asperomantis* species by high distances of 3.3–9.3% and are phylogenetically distinct from other *Asperomantis*. It is thus clear that the current taxonomy requires revision and the bioacoustic and genetic distinctness of these frogs needs to be carefully evaluated.

Taxonomic conclusions

Genetically, specimens from Ampotsidy+Antsahan'i Ledy and Andranonafindra+Bealanana-Antsohihy are separated by 1–3% in the segment of the 16S rRNA gene typically used for candidate species designation

in Madagascan amphibians (Vieites et al. 2009). This is relatively low, and the typical threshold for establishment of candidate species (3% uncorrected p-distance) is barely achieved. However, bioacoustics tells a different story: the differences in the sound of the call (tonal and unpulsed vs. noisy and pulsed; fairly short, isolated series vs. long, ill-defined series) are remarkable. Morphological data among these populations are equivocal; although males from Ampotsidy are smaller than those from Andranonafindra and the dimensions of their femoral glands differ somewhat, all other characters show no distinction among the populations, albeit with low sample

Table 2. Uncorrected pairwise distances among members of the subfamily *Asperomantis* in the *16S* marker; the diagonal values refer to intra-specific distinction. For uncorrected p-distances for the whole genus *Gephyromantis*, see Supplementary material 6.

	1	2	3	4	5	6	7
1. <i>Gephyromantis</i> sp. Ca29	0.0%						
2. <i>Gephyromantis angano</i> sp. n.	1.0–3.0%	0.0–0.2%					
3. <i>Gephyromantis tahotra</i>	6.5–8.9%	7.3–9.3%	0.0–3.3%				
4. <i>Gephyromantis ceratophrys</i>	6.1–6.5%	6.0–6.7%	6.9–8.2%	0.0%			
5. <i>Gephyromantis asper</i>	3.3–4.5%	3.9–5.9%	6.1–8.6%	7.1–7.8%	0.0–1.4%		
6. <i>Gephyromantis spinifer</i>	4.9–6.7%	5.3–7.5%	7.9–9.4%	7.6–7.8%	5.2–7.3%	0.6–3.1%	
7. <i>Gephyromantis ambohitra</i>	6.7–8.5%	7.4–9.3%	11.5–14.8%	11.0–11.3%	9.6–11.7%	9.7–11.8%	0.0–7.1%

sizes. This is typical of some *Asperomantis* species however, and a similar lack of morphological difference is to be found between *G. asper* and *G. ceratophrys* (Vences et al. 2017).

In summary, evidence from mitochondrial DNA, bioacoustics, and morphology currently suggests a weak degree of differentiation between these two populations, with the greatest differences being in sound and structure of the advertisement calls. It may thus be possible that both of these forms represent separate species. We therefore assign the populations from Andranonafindra and Bealanana-Antsohihy a new candidate species number, *Gephyromantis* sp. Ca29, and consider it an Unconfirmed Candidate Species sensu Vieites et al. (2009). It is apparent however that specimens from Antsahan'i Ledy and Ampotsidy, representing *G.* sp. Ca28 in the original sense (Perl et al. 2014), are distinct from all currently described *Gephyromantis* species. We therefore describe this form as a new species in the following. Whether *G.* sp. Ca29 indeed represents an independent evolutionary lineage also meriting formal description, or if it is better seen as deep conspecific lineage of the new species described here, can only be decided with further genetic and field data.

Gephyromantis (Asperomantis) angano sp. n.

<http://zoobank.org/B1DA196D-21E4-4A45-9D4A-B6E8DBF912F6>
Figures 1, 3–7, Suppl. material 1, 2, 5

Gephyromantis sp. Ca28 — Perl et al. (2014)

Holotype. ZSM 68/2016 (MSZC 0172), an adult male, collected at 22h40 on 8th January 2016 in Ampotsidy (14.41949°S, 48.71938°E, 1340 m a.s.l.), roughly 15 km north of Bealanana in the Bealanana District, Sofia Region, northern Madagascar, by Mark D. Scherz, James Borrell, Lawrence Ball, Thomas Starnes, Elidiot Razafimandimby, Denise Herizo Nomenjanahary, and Jeanne-ney Rabearivony.

Paratypes. ZSM 67/2016 (MSZC 0112) adult female, collected at night on 30th December 2015 in Ampotsidy (14.41734°S, 48.71858°E, 1363 m a.s.l.); ZSM 69/2016 (MSZC 0021) adult male, collected at 22h00 on 19th December 2015 in Ampotsidy (14.41956°S, 48.71946°E, 1357 m a.s.l.); UADBA-A uncatalogued (MSZC 0032)

adult male, collected in the late morning on the 21st December 2015 in Ampotsidy (14.41435°S, 48.71155°E, 1431 m a.s.l.); UADBA-A uncatalogued (MSZC 0053) subadult, collected at 20h43 on 22nd December 2015 in Ampotsidy (14.41382°S, 48.71178°E, 1443 m a.s.l.); UADBA-A uncatalogued (MSZC 0091), an adult female collected at night on 30th December 2015 in Ampotsidy (14.41208°S, 48.71609°E, 1513 m a.s.l.); all collected by Mark D. Scherz, James Borrell, Lawrence Ball, Thomas Starnes, Elidiot Razafimandimby, Denise Herizo Nomenjanahary, and Jeanneney Rabearivony. ZSM 1731/2010 (ZCMV 12303), adult female, collected on 9th June 2010 on the Tsaratanana massif, in the forest near camp 0 (Antsahan'i Ledy; 14.2332°S, 48.9800°E, 1207 m a.s.l.) by Miguel Vences, David R. Vieites, Roger-Daniel Randrianiaina, Fanomezana Ratsoavina, Solohery Rasamison, Andolalao Rakotoarison, Emile Rajeriarison, and Theo Rajoafiarison.

Diagnosis. A *Gephyromantis* species assigned to the subgenus *Asperomantis* based on the presence of small dermal spines on the elbow and heel, presence of inner and outer dorsal ridges as defined by Vences and Glaw (2001), Type 2 femoral glands sensu Glaw et al. (2000)Glaw et al. (2000), moderately enlarged finger and toe tips, absence of webbing between fingers, moderate webbing between toes, presence of paired blackish sub-gular vocal sacs in males, and a distinct whitish spot in the middle of the tympanic field (Vences et al. 2017). DNA sequence data from a fragment of the *16S* gene supports this assignment. *Gephyromantis angano* sp. n. is characterized by the following suite of morphological characters: (1) adult SVL 29.1–30.5 mm, (2) TD/ED 0.61–0.71, (3) small supraocular spines, (4) large femoral glands consisting of numerous small granules, (5) moderately raised dorsal ridges, (6) granular dorsal skin, (7) relatively short hindlimbs (HIL/SVL 1.73–1.81 in males), and (7) its unique call (see above).

Within the subgenus *Asperomantis*, *Gephyromantis angano* sp. n. can be distinguished from *G. ambohitra*, *G. spinifer*, and *G. tahotra* by its smaller size (male SVL < 30 mm, vs. >31 mm, female SVL up to 30.5 mm vs. >32 mm); from *G. spinifer* by its less granular dorsal skin and smaller supraocular spines; from *G. asper* and *G. ceratophrys* by its generally shorter hindlimbs in males (HIL/SVL 1.73–1.81 vs. 1.77–2.11); and from *G. ceratophrys* by more granules per femoral gland (26–69 vs. 14–20).

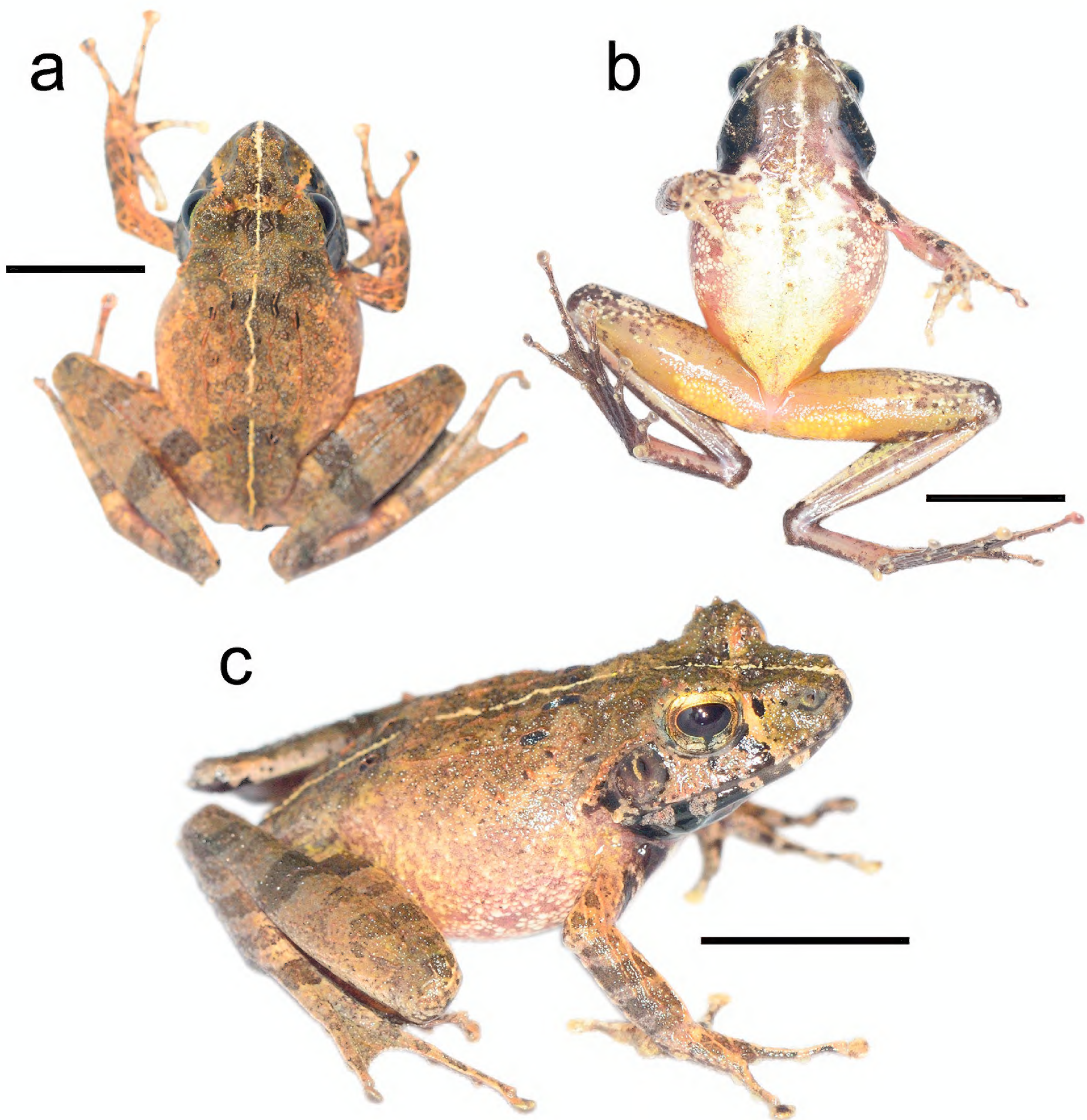


Figure 5. The holotype of *Gephyromantis angano* sp. n., ZSM 68/2016 (MSZC 0172) in life in (a) dorsal, (b) ventral, and (c) lateral view. Scale bars indicate 10 mm.

Bioacoustically, it is distinguished from all of these species by its call duration (41–98 ms vs. 5–44 ms in *G. asper* and *G. ceratophrys*, and 98–274 ms in *G. ambohitra* and *G. tahotra*), unpulsed calls (vs. pulsed in *G. ambohitra* and *G. tahotra*), calls repeated faster than in *G. ceratophrys*, and dominant frequency (3703–3875 Hz vs. 1435–3366 Hz in *G. ambohitra*, and *G. tahotra*).

Description of the holotype. A specimen in a good state of preservation, the left thigh cut for DNA tissue sample and to expose the inner face of the femoral gland. Snout-vent length 29.6 mm. For other measurements see Table 1. Body rather rounded; head longer than wide, as wide as the body; snout acuminate in dorsal view, truncate in lateral

view; nostrils directed laterally, slightly protuberant, much nearer to tip of snout than to eye; canthus rostralis distinct, concave; loreal region concave and moderately oblique; tympanum distinct, round, its diameter 71% of eye diameter; supratympanic fold distinct, curving ventrally; tongue ovoid, distinctly bifid posteriorly; vomerine teeth distinct, in two small aggregations, positioned posteromedially to choanae; choanae rounded. Dark dermal fold (the inflatable parts of the vocal sacs) running along each lower jaw from commissure of mouth to middle of lower jaw. Arms slender, subarticular tubercles single; outer metacarpal tubercle very poorly developed and inner metacarpal tubercle relatively well developed; fingers without webbing; relative length of fingers $1 < 2 < 4 < 3$, second finger dis-

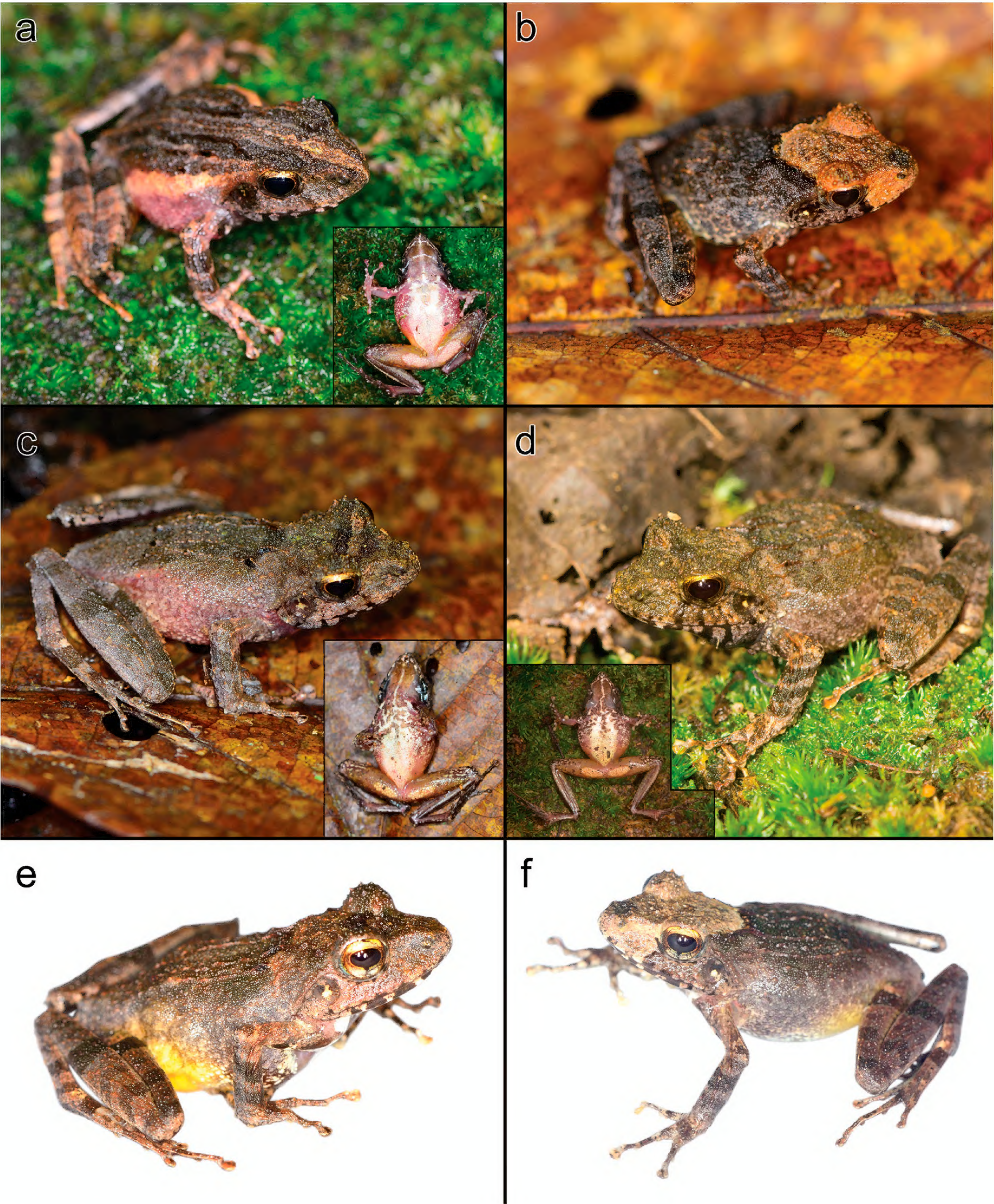


Figure 6. Variation in *Gephyromantis angano* sp. n. (a) UADBA-A uncatalogued (MSZC 0032), adult male (FGG = 69/56), (b) UADBA-A uncatalogued (MSZC 0053), juvenile, (c) ZSM 67/2016 (MSZC 0021), adult male (FGG = 30/26), (d) UADBA-A uncatalogued (MSZC 0091), adult male (FGG = 57/55), (e) Université d’Antsiranana uncatalogued (MSZC 0088), adult female (not in the type series), (f) ZSM 69/2016 (MSZC 0112), adult female. Insets show specimens in ventral view. Not to scale.

tinctly shorter than fourth; finger discs distinctly enlarged, nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaching beyond snout tip when hindlimb is adpressed along body; lateral metatarsals separated by webbing; in-

ner metatarsal tubercle distinct, outer metatarsal tubercle very faint but present; webbing formula of foot according to Blommers-Schlösser (1979) 1(1), 2i(1.5), 2e(1), 3i(2), 3e(1), 4i(2.5), 4e(2), 5(0.5); relative toe length $1 < 2 < 5$

$< 3 < 4$; toe discs distinctly enlarged. Skin dorsally granular; ridges bordering mid-dorsal band elevated, starting approximately 1 mm behind eyes (starting off bifurcated and converging toward the mid-line) and gradually becoming less distinct posteriorly; additional, interrupted and less distinct ridges are present posterior to the suprascapular region; two dark inter-ocular ridges are present either side of a fine cream-coloured vertebral band; supraocular tubercles are weakly enlarged, and do not form strong spines above the eyes; a modest dermal tarsal spine is present. Ventral skin smooth on throat and limbs, granular in posterior portion of abdomen. Femoral glands well delimited externally, consisting of 36 small granules on the left side and 44 small granules on the right side.

Dorsal colouration after one and a half years in preservative sepia, becoming increasingly grey posteriorly, mottled with almost black and brownish markings; dorsal folds are blackened over the suprascapular region but are otherwise brown; the tympanum is darker brown than the surrounding area; the lateral head has a cream stripe before the eye, immediately followed by a black stripe roughly 1 mm wide, and then mottled dark and light until the tympanum; bottom lip has alternating brown and cream annulations; dorsal forelimbs mottled blackish and Mikado brown reticulated with cream; dorsal hindlimbs brown with burnt umber crossbands on the thigh (three), shank (four), and foot (four); the cloacal region has a trapezoid of burnt umber around it; flank colouration fades from the sepia dorsal colouration through grey to the cream of the venter; ventrally the chin is medium fawn with a cream mid-ventral stripe and blackish vocal sacs, becoming blotched fawn among cream posteriorly to fully cream on the abdomen; the ventral legs are cream with brown and black areas toward the knees and on the anteroventral edge of the shank, including the femoral glands, which are distinct only in their texture and shape, and not in colour; the ventral foot is dark brown.

Colouration in life was as in preservative but more vibrant; see Figure 5.

Variation. For a summary of measurement variation, see Table 1. All morphologically examined paratypes strongly resemble the holotype in morphology. Ridges between the eyes vary somewhat in shape, and in some specimens are black but in others do not have a distinct colouration from the surrounding head surface. The dorsal ridges vary from strongly to weakly pronounced, but are always present. There is no sexual dimorphism in inner metacarpal tubercle size. Snout shape in lateral view varies from rounded to square. The superciliary spines of all specimens are fairly low and indistinct. The femoral glands are remarkably variable, ranging from 26 granules in the right gland of ZSM 69/2016 to 69 granules in the left gland of MSZC 0032 (Fig. 6). Variation in colouration is as variable as is typical for members of this subgenus. A thin vertebral line can be present. The arms are always reticulated with whitish to light brown colouration. The head of ZSM 69/2016 (Fig. 6f) has a diamond-shaped lighter colouration

covering its dorsal surface. The ventral colouration of this specimen is remarkably similar to that of all males, except that the blackish vocal sacs are absent. A juvenile, MSZC 0032, also had this diamond-shaped brown marking on its head (Fig. 6b).

Etymology. *Angano* is a Malagasy word meaning ‘fable’. The new material for this species was collected on Expedition Angano, a research expedition to the Bealanana District of northern Madagascar to assess the impacts of forest fragmentation on the reptiles and amphibians. The epithet is used as an invariable noun in apposition to the genus name.

Call. See the description provided above.

Natural history and distribution. One specimen of this species has been collected in Antsahan’i Ledy, and numerous specimens of this species were encountered during fieldwork on the Ampotsidy mountains (Fig. 7). Calling males were generally found in association with slow flowing water, in the case of the holotype at the source of a spring, in close syntopy with *Boophis madagascariensis* and a *Mantidactylus* (*Brygoomantis*) species. Males called up to 1 m above the ground from fern fronds and other low foliage. Females were found both near to and away from water, during the day and at night, but were less commonly encountered. No eggs were observed, but highly ovigerous females were found in January (e.g. Fig. 6e). The call of the species is loud and carries over long distances, so that it can be heard alongside the calls of *Boophis madagascariensis* from well outside of some small forest fragments in the vicinity of Ampotsidy. In a small forest fragment where vouchers of *Gephyromantis* (*Asperomantis*) *tahotra* were collected (14.41689°S, 048.71435°E, 1368 m a.s.l.), *G. angano* sp. n. could also be heard; this appears to be the first ever record of any two *Asperomantis* species occurring in close syntopy (Vences et al. 2017).

Discussion

Gephyromantis is one of the most diverse genera of frogs in Madagascar. Since the first major barcoding study of all of Madagascar’s amphibians in 2009 (Vieites et al. 2009), five species of *Gephyromantis* have been described (Crotini et al. 2011, Glaw et al. 2011, Glaw and Vences 2011, Vieites et al. 2012, Wollenberg et al. 2012), two have been resurrected (Wollenberg et al. 2012, Vences et al. 2017), and numerous undescribed species remain, including two that are in description (Scherz et al. in press; submitted). During our fieldwork in the Bealanana District of northern Madagascar, we encountered a total of six *Gephyromantis* species verified by DNA barcoding (Suppl. material 5), including three in the subgenus *Asperomantis* (*G. angano* sp. n., *G. sp.* Ca29 [new; Fig. 8] and *G. tahotra*, Fig. 4), one in the subgenus *Laurentomantis* (identified as *G. horridus*, but separated from other *G. horridus* by 3% 16S divergence and requiring closer investigation), one in the

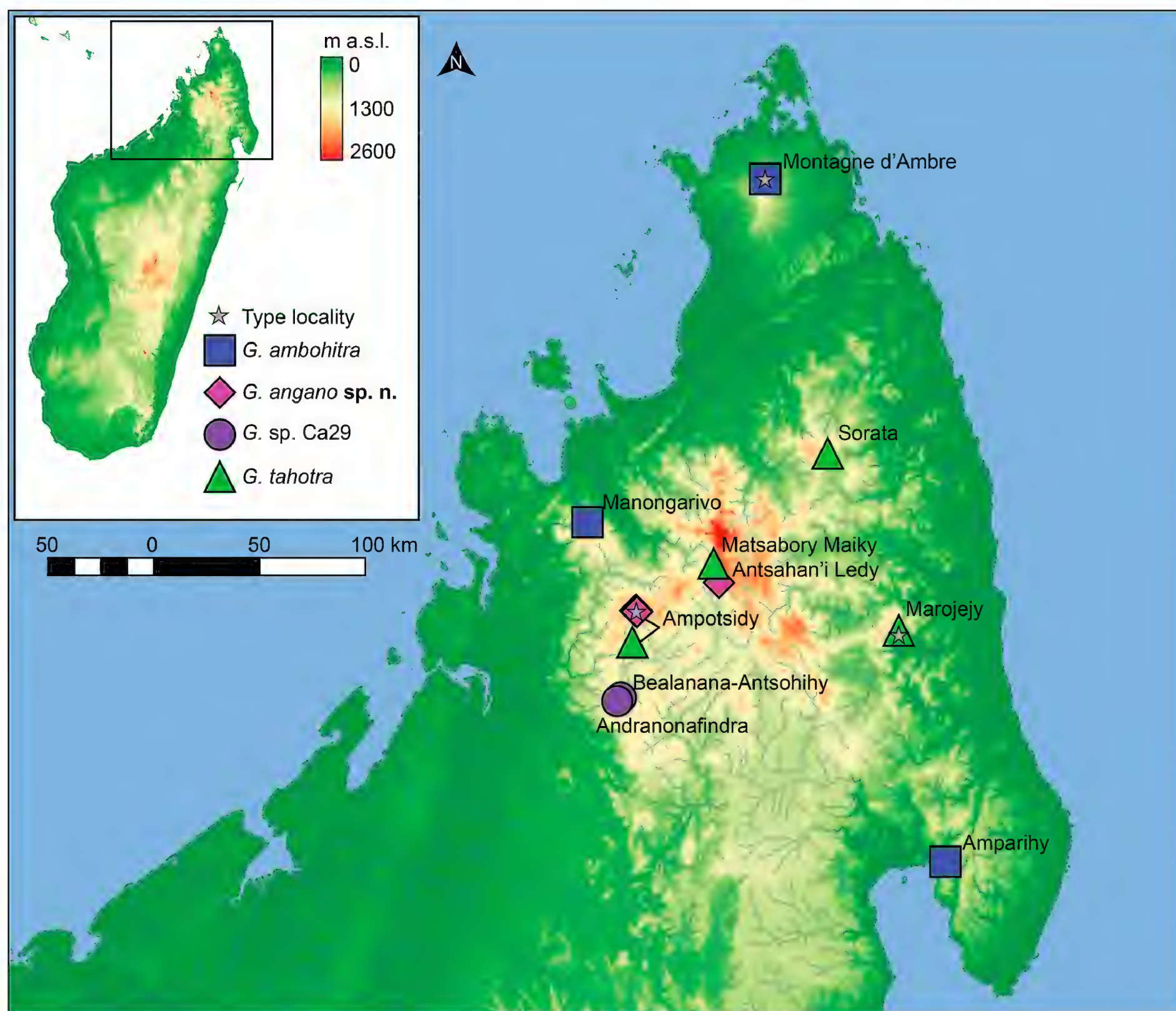


Figure 7. Map of northern Madagascar indicating the known distribution of *Asperomantis* species. Colours correspond to species in Fig. 4. Three arc second SRTM basemap from Jarvis et al. (2008).

subgenus *Duboimantis* (a new candidate species close to *G. tandroka*, here dubbed *G. sp. Ca30*), and one in the subgenus *Phylacomantis* (a new candidate species close to *G. azzurrae*, here dubbed *G. sp. Ca31*) (see Suppl. material 5). Of these, only two (*G. tahotra* and *G. horridus*) are already described, and Ampotsidy represents a new locality for both of them. The new Unconfirmed Candidate Species of *Duboimantis* and *Phylacomantis* are numbered and rationalised following Vieites et al. (2009). In summary, although recent advances have brought major improvements to the supraspecific taxonomy of *Gephyromantis* (Kaffenberger et al. 2012, Vences et al. 2017), work on the species-level taxonomy of the genus is far from finished.

Several hypotheses may be put forward to explain the differences between *G. angano* sp. n. and its bioacoustically divergent but genetically and morphologically similar sister lineage *G. sp. Ca29* (shown in Fig. 8): (1) these represent two call types for the same species, as is known from the closely related *G. tahotra*, and also *Boophis tampoka*, a tree frog from Madagascar that also has two

call types that are not genetically assortative and change by locality or temporally (Vences et al. 2011) (considered unlikely, as the calls were heard within five days of one another at the two sites, and neither call was ever heard at both sites despite numerous calling individuals being observed, and over three weeks of observations from one of the sites), (2) the calls form two ends of a continuum of variation over the distribution of a single species (seemingly unlikely, given they are correlated with genetic differences and are rather important, affecting several call variables), (3) the calls represent local dialects caused by a slight structural modification of the vocal apparatus, (4) the two populations are undergoing incipient speciation or (5) they are two distinct, recently diverged species. In frogs, advertisement calls play a strong role in sexual selection and mate recognition (Hoskin et al. 2005, Köhler et al. 2017), and call differences may function as drivers and/or reinforcers of divergence (Hoskin et al. 2005). In either case, they can evolve exceptionally quickly, in a way that can greatly exceed signals of typical drift-based

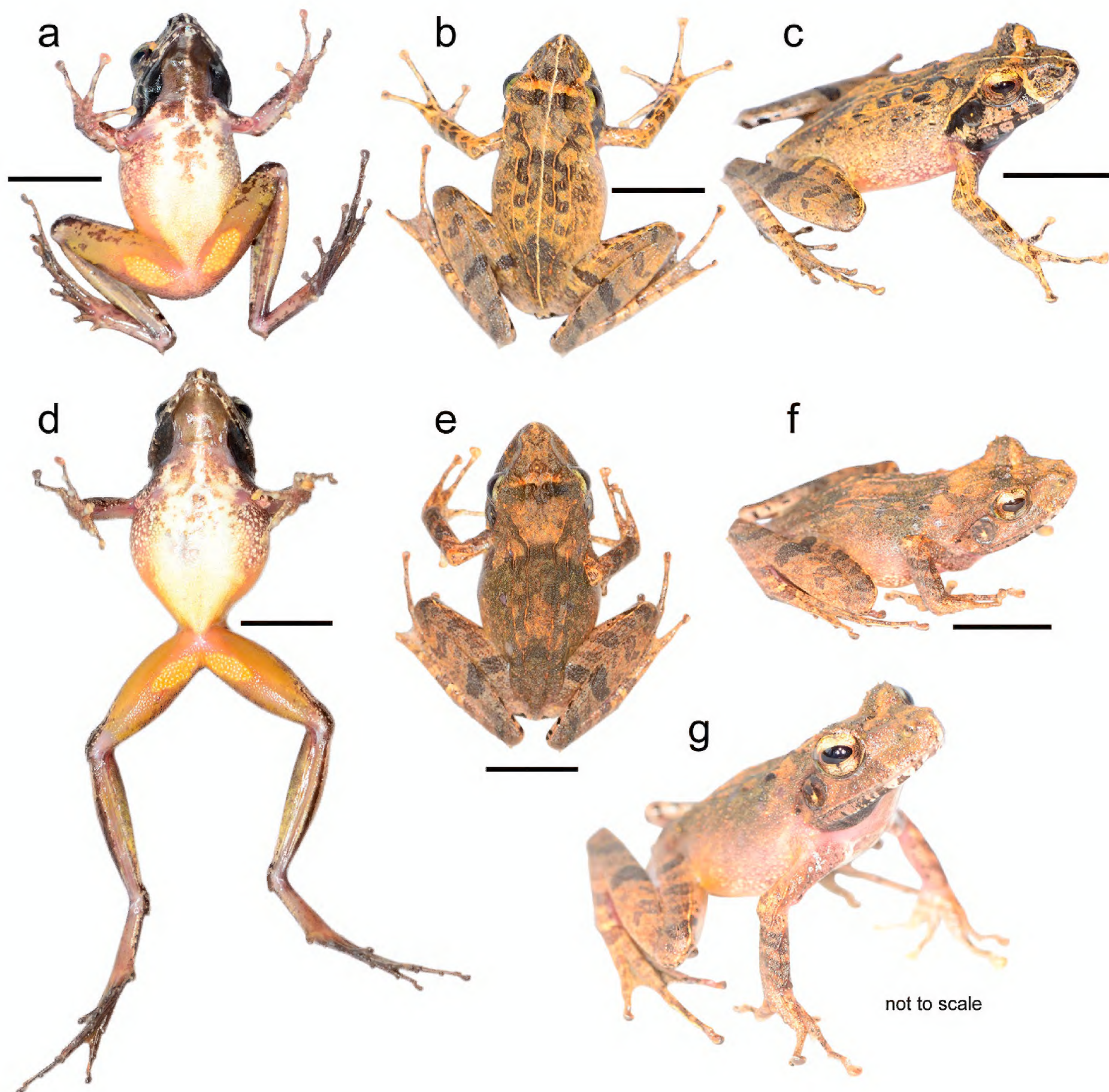


Figure 8. *Gephyromantis* sp. Ca29 from Andranonafindra in life. Photos show (a–c) ZSM 59/2016 (MSZC 0203), adult male (FGG = 48/47), and (e–g) ZSM 58/2016 (MSZC 0196), adult male (FGG = 42/49). Scale bars indicate 10 mm.

divergence, which can lead to cases where signals from mitochondrial genes simply have not yet caught up, but are likely to do so. With greater sampling and sequencing of nuclear genes, we may be able to reveal which of these hypotheses is most credible, but at present data are insufficient to draw convincing conclusions on this matter. Denser sampling across a greater area of the Bealanana basin to identify possible contact or hybrid zones will also be critical in understanding the divergence pattern and phylogeography of these frogs, but such work will undoubtedly be challenging, given the extreme fragmentation of forests in this area, and how difficult it is for research teams to access its more remote reaches.

The new species *Gephyromantis angano* sp. n. is restricted to primary and secondary mid-altitude rainforest

(Fig. 7). These forests are disappearing rapidly in the Bealanana District, becoming increasingly fragmented, with fragments decreasing in size. Based on bioacoustic surveys while crossing between large forest fragments, it seems that *G. angano* sp. n. is able to survive in even tiny forest remnants, but a degree of connectivity is doubtless required in order to facilitate gene flow. At present the two known localities make up an area of just 90 km², most of which is devoid of forest. As such, the species could warrant treatment as Critically Endangered. However, due to its relative abundance, and because we suspect that it is more widespread, we recommend that it instead be assessed as Data Deficient for the IUCN Red List until better sampling in the Bealanana District can be carried out.

Acknowledgements

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Supplementary material 1

Recording 1

Authors: Mark D. Scherz, Miguel Vences, James Borrell, Lawrence Ball, Denise Herizo Nomenjanahary, Duncan Parker, Marius Rakotondratsima, Elidiot Razafimandimby, Thomas Starnes, Jeanneney Rabearivony, Frank Glaw

Data type: FLAC File (.flac)

Explanation note: Call recording of *Gephyromantis angano* sp. n. ZSM 68/2016. Call recorded at 22h40 on the 8th of January, 2016, 50 cm above the ground on a fern above a muddy spring in primary rainforest, calling as part of a small chorus, at 14.41949°S, 48.71938°E, 1340 m a.s.l., at an estimated air temperature between 15 and 20°C. Animal Sound Archive: http://www.tierstimmenarchiv.de/webinterface/contents/showdetails.php?edit=-1&unique_id=TSA:Gephyromantis_angano_Scherz_1_1_0&autologin=true

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Link: <https://doi.org/10.3897/zse.93.14906.suppl1>

Supplementary material 2

Recording 2

Authors: Mark D. Scherz, Miguel Vences, James Borrell, Lawrence Ball, Denise Herizo Nomenjanahary, Duncan Parker, Marius Rakotondratsima, Elidiot Razafimandimby, Thomas Starnes, Jeanneney Rabearivony, Frank Glaw

Data type: WAV File (.wav)

Explanation note: Call recordings of *Gephyromantis angano* sp. n. uncollected specimens. Call recorded at ca. 03h30 on the 8th of January, 2016 near a muddy spring in primary rainforest, at 14.41949°S, 48.71938°E,

1340 m a.s.l. Animal Sound Archive: http://www.tierstimmenarchiv.de/webinterface/contents/showdetails.php?edit=-1&unique_id=TSA:Gephyromantis_angano_Scherz_1_2_0&autologin=true

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Link: <https://doi.org/10.3897/zse.93.14906.suppl2>

Supplementary material 3

Recording 3

Authors: Mark D. Scherz, Miguel Vences, James Borrell, Lawrence Ball, Denise Herizo Nomenjanahary, Duncan Parker, Marius Rakotondratsima, Elidiot Razafimandimby, Thomas Starnes, Jeanneney Rabearivony, Frank Glaw

Data type: WAV File (.wav)

Explanation note: Call recording of *Gephyromantis* sp. Ca29 ZSM 58/2016. Call recorded at 18h40 on the 14th of January, 2016 on a broad fleshy leaf 4 m from a slow stream in degraded primary rainforest, calling as part of a large chorus, at 14.73600°S, 48.54831°E, 1180 m a.s.l., at an estimated air temperature of 17–23°C. Animal Sound Archive: http://www.tierstimmenarchiv.de/webinterface/contents/showdetails.php?edit=-1&unique_id=TSA:Gephyromantis_sp_Ca_29_Scherz_1_3_0&autologin=true

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Link: <https://doi.org/10.3897/zse.93.14906.suppl3>

Supplementary material 4

Recording 4

Authors: Mark D. Scherz, Miguel Vences, James Borrell, Lawrence Ball, Denise Herizo Nomenjanahary, Duncan Parker, Marius Rakotondratsima, Elidiot Razafimandimby, Thomas Starnes, Jeanneney Rabearivony, Frank Glaw

Data type: WAV File (.wav)

Explanation note: Call recording of *Gephyromantis* sp. Ca29 ZSM 59/2016. Call recorded at 18h25 on the 14th of January, 2016 on a leaf 50 cm above ground several metres from a slow stream in degraded primary rainforest, calling as part of a large chorus, at 14.73600°S, 48.54831°E,

1180 m a.s.l., at an estimated air temperature of 17–23°C. Animal Sound Archive: http://www.tierstimmenarchiv.de/webinterface/contents/showdetails.php?edit=-1&unique_id=TSA:Gephyromantis_sp_Ca_29_Scherz_1_4_0&autologin=true

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Link: <https://doi.org/10.3897/zse.93.14906.suppl4>

Supplementary material 5

Figure S1

Authors: Mark D. Scherz, Miguel Vences, James Borrell, Lawrence Ball, Denise Herizo Nomenjanahary, Duncan Parker, Marius Rakotondratsima, Elidiot Razafimandimby, Thomas Starnes, Jeanneney Rabearivony, Frank Glaw

Data type: Encapsulated PostScript (.eps)

Explanation note: Phylogeny of *Gephyromantis* based on the BI consensus tree reconstructed by Bayesian Inference analysis of a fragment of the mitochondrial 16S rRNA gene. Numbers above nodes denote Bayesian Posterior Probability (PP); numbers below nodes indicate bootstrap support (%). PP lower than 0.9 and bootstrap support lower than 70% are not shown.

Numbers before taxon names are GenBank numbers; numbers after taxon names are field numbers.

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Link: <https://doi.org/10.3897/zse.93.14906.suppl5>

Supplementary material 6

Table S1

Authors: Mark D. Scherz, Miguel Vences, James Borrell, Lawrence Ball, Denise Herizo Nomenjanahary, Duncan Parker, Marius Rakotondratsima, Elidiot Razafimandimby, Thomas Starnes, Jeanneney Rabearivony, Frank Glaw

Data type: Microsoft Excel 97-2003 Worksheet (.xls)

Explanation note: Average uncorrected pairwise-distances in a fragment of the 16S rRNA gene among *Gephyromantis* species.

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Link: <https://doi.org/10.3897/zse.93.14906.suppl6>